MOLECULAR BIOLOGY OF
THE CELL
THIRD EDITION

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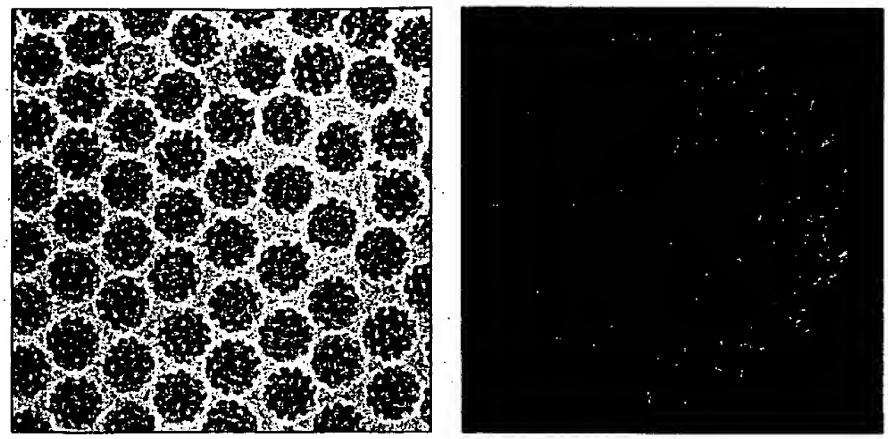
Front cover: The photograph shows a rat nerve cell in culture. It is labeled (*yellow*) with a fluorescent antibody that stains its cell body and dendritic processes. Nerve terminals (*green*) from other neurons (not visible), which have made synapses on the cell, are labeled with a different antibody. (Courtesy of Olaf Mundigl and Pietro de Camilli.)

Dedication page: Gavin Borden, late president of Garland Publishing, weathered in during his mid-1980s climb near Mount McKinley with MBoC author Bruce Alberts and famous mountaineer guide Mugs Stump (1940–1992).

Back cover: The authors, in alphabetical order, crossing Abbey Road in London on their way to lunch. Much of this third edition was written in a house just around the corner. (Photograph by Richard Olivier.)



(A) (B)

Figure 4–30 **Electron microscopy of a virus.** (A) Unstained Semliki forest virus in a thin layer of vitrified water viewed by cryoelectron microscopy at −160°C. As in light microscopy, phase contrast can be used to get an image of the unstained specimen. A large number of these images can then be combined by image-processing methods to produce a three-dimensional image of the virus at high resolution (B). (Courtesy of Stephen Fuller.)

shape of an individual protein molecule to the remarkable resolution of 0.35 nm (see Figure 10–31). But even in its most sophisticated forms, electron microscopy falls short of providing a full description of molecular structure because the atoms in a molecule are separated by distances of only 0.1 or 0.2 nm. Resolving molecular structure in atomic detail takes us beyond microscopy to techniques such as x-ray diffraction, which are described in a later section.

Summary

Many light-microscope techniques are available for observing cells. Cells that have been fixed and stained can be studied in a conventional light microscope, while antibodies coupled to fluorescent dyes can be used to locate specific molecules in cells in a fluorescence microscope. The confocal scanning microscope provides thin optical sections and can be used to reconstruct a three-dimensional image. Living cells can be seen with phase-contrast, differential-interference-contrast, or dark-field optics. All forms of light microscopy are facilitated by electronic image-processing techniques, which enhance sensitivity and refine the image.

Determining the detailed structure of the membranes and organelles in cells requires the higher resolution attainable in a conventional transmission electron microscope. Three-dimensional views of the surfaces of cells and tissues can be obtained by scanning electron microscopy, while the interior of membranes and cells can be visualized by freeze-fracture and freeze-etch electron microscopy, respectively. The shapes of isolated macromolecules that have been shadowed with a heavy metal or outlined by negative staining can also be readily visualized by electron microscopy.

Isolating Cells and Growing Them in Culture [14]

Although the structure of organelles and large molecules in a cell can be seen with microscopes, a molecular understanding of a cell requires detailed biochemical analysis. Unfortunately, most biochemical procedures require large numbers of cells and begin by disrupting them. If the sample is a piece of tissue, fragments of all of its cells will be mixed together, creating confusion if the cells are of several types, which is almost always the case. In order to preserve as much information as possible about each individual type of cell, cell biologists have developed ways of dissociating cells from tissues and separating the various types. The resulting, relatively homogenous population of cells then can be analyzed—either directly or after their number has been greatly increased by allowing them to proliferate in culture.

Cells Can Be Isolated from a Tissue and Separated into Different Types [15]

The first step in isolating cells of a uniform type from a tissue that contains a mixture of cell types is to disrupt the extracellular matrix and intercellular junctions that hold the cells together. The best yields of viable dissociated cells are usually obtained from fetal or neonatal tissues, typically by treating them with proteolytic enzymes (such as trypsin and collagenase) and with agents (such as ethylenediaminetetraacetic acid, or EDTA) that bind, or *chelate,* the Ca^{2+} on which cell-cell adhesion depends. The tissue can then be dissociated into single viable cells by gentle agitation.

Several approaches are used to separate the different cell types from a mixed cell suspension. One involves exploiting differences in physical properties. Large cells can be separated from small cells and dense cells from light cells by centrifugation, for example. These techniques will be described in connection with the separation of organelles and macromolecules, for which they were originally developed. Another approach is based on the tendency of some cell types to adhere strongly to glass or plastic, which allows them to be separated from cells that adhere less strongly.

An important refinement of this last technique depends on the specific binding properties of antibodies. Antibodies that bind specifically to the surface of only one cell type in a tissue can be coupled to various matrices—such as collagen, polysaccharide beads, or plastic—to form an *affinity surface* to which only cells recognized by the antibodies will adhere. The bound cells are then recovered by gentle shaking, by treatment with trypsin to digest the proteins that mediate the adhesion, or, in the case of a digestible matrix (such as collagen), by degrading the matrix itself with enzymes (such as collagenase).

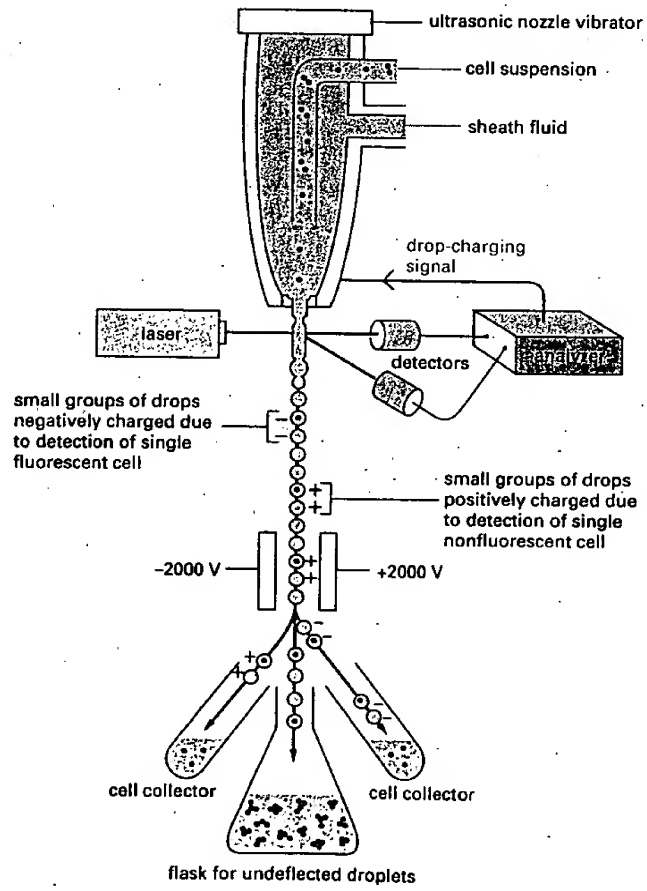


Figure 4–31 A fluorescence-activated cell sorter. When a cell passes through the laser beam, it is monitored for fluorescence. Droplets containing single cells are given a negative or positive charge, depending on whether the cell is fluorescent or not. The droplets are then deflected by an electric field into collection tubes according to their charge. Note that the cell concentration must be adjusted so that most droplets contain no cells and flow to a waste container together with any cell clumps. The same apparatus can also be used to separate fluorescently labeled chromosomes from one another, providing valuable starting material for the isolation and mapping of genes.

The most sophisticated cell-separation technique involves labeling specific cells with antibodies coupled to a fluorescent dye and then separating the labeled cells from the unlabeled ones in an electronic **fluorescence-activated cell sorter.** Here, individual cells traveling in single file in a fine stream are passed through a laser beam and the fluorescence of each cell is measured. Slightly farther downstream, tiny droplets, most containing either one cell or no cells, are formed by a vibrating nozzle. The droplets containing a single cell are automatically given a positive or a negative charge at the moment of formation, depending on whether the cell they contain is fluorescent; they are then deflected by a strong electric field into an appropriate container. Occasional clumps of cells, detected by their increased light scattering, are left uncharged and are discarded into a waste container. Such machines can select 1 cell in 1000 and sort about 5000 cells each second (Figure 4–31).

When a uniform population of cells has been obtained by any of these methods, it can be used directly for biochemical analysis. Alternatively, it provides a suitable starting material for cell culture, allowing the complex behavior of cells to be studied under the strictly defined conditions of a culture dish.

Cells Can Be Grown in a Culture Dish [16]

Given appropriate conditions, most kinds of plant and animal cells will live, multiply, and even express differentiated properties in a tissue-culture dish. The cells can be watched under the microscope or analyzed biochemically, and the effects of adding or removing specific molecules, such as hormones or growth factors, can be explored. In addition, in a mixed culture the interactions between one cell type and another can be studied. Experiments on cultured cells are sometimes said to be carried out *in vitro* (literally, "in glass") to contrast them with experiments on intact organisms, which are said to be carried out *in vivo* (literally, "in the living organism"). The terms can be confusing because they are often used in a different sense by biochemists, for whom *in vitro* refers to biochemical reactions occurring outside living cells, while *in vivo* refers to any reaction taking place inside a living cell.

Tissue culture began in 1907 with an experiment designed to settle a controversy in neurobiology. The hypothesis under examination was known as the *neuronal doctrine,* which states that each nerve fiber is the outgrowth of a single nerve cell and not the product of the fusion of many cells. To test this contention, small pieces of spinal cord were placed on clotted tissue fluid in a warm, moist chamber and observed at regular intervals under the microscope. After a day or so, individual nerve cells could be seen extending long, thin processes into the clot. Thus the neuronal doctrine was validated, and the foundations for the cell-culture revolution were laid.

The original experiments in 1907 involved the culture of small tissue fragments, or **explants.** Today, cultures are more commonly made from suspensions of cells dissociated from tissues as described above. Unlike bacteria, most tissue cells are not adapted to living in suspension and require a solid surface on which to grow and divide, which is now usually the surface of a plastic tissue-culture dish (Figure 4–32). Cells vary in their requirements, however, and some will not grow or differentiate unless the culture dish is coated with specific extracellular matrix components, such as collagen or laminin.

Cultures prepared directly from the tissues of an organism, either with or without an initial cell-fractionation step, are called **primary cultures.** In most cases cells in primary cultures can be removed from the culture dish and used to form a large number of **secondary cultures;** they may be repeatedly subcultured in this way for weeks or months. Such cells often display many of the differentiated properties appropriate to their origin: fibroblasts continue to secrete

Figure 4–32 Cells in culture. Scanning electron micrograph of rat fibroblasts growing on the plastic surface of a tissue-culture dish. (Courtesy of Guenter Albrecht-Buehler.)